Atomic Force Microscopy

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Tip Trajectory Mapping in Atomic Force Microscopy

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Atomic Force Microscopy (AFM) is a powerful technique for probing biological macromolecules. In conventional AFM, knowledge of the local 3D tip position is not accessible; tip trajectories are inferred from cantilever deflection which supplies data of reduced dimensionality. Previously, we developed an ultrastable AFM (US-AFM) with atomic-scale tip-sample stability and registration in room temperature air. We have recently extended US-AFM to operate in fluid and in dynamic (e.g., ''tapping'') mode, enabling precise studies of soft biological samples in their native environments. With the local tip position detection inherent in US-AFM, we have observed the trajectories of an AFM tip in 3D as it interacts with a sample surface in both tapping and contact modes in fluid. These observations provide a more complete picture of local tip dynamics during imaging and force spectroscopy.

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Improving Single Molecule Force Spectroscopy through Automated Real-Time Data Collection and Quantification of Experimental Conditions

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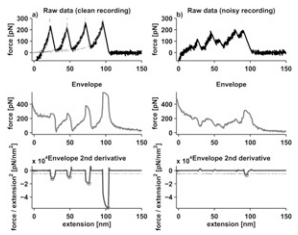
The benefits of single molecule force spectroscopy (SMFS) clearly outweigh the challenges which include small sample sizes, tedious data collection and introduction of human bias during the subjective data selection. These difficulties can be partially eliminated through automation of the experimental data collection process for atomic force microscopy (AFM). Automated data collection can also provide information about the frequency of successful force extension recordings. Here, we report a methodology for automation of the real-time data collection for AFM for SMFS. This method uses a Hilbert transform to calculate the force-extension envelope and then calculates the sharpness of the force rupture events to discern usable recordings (Figure 1A) from noisy recordings (Figure 1B) in real-time.



We implemented this methodology in our laboratory and used the statistics provided by the automated procedure to clearly demonstrate the properties of molecular adhesion and how these properties change with differences in the cantilever tip and protein functional groups and protein age. We find evidence that a terminal cysteine residue on a protein and an Au-coated cantilever tip increases the proportion of full length molecule recordings.

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Spiral Scanning and Encased Cantilevers for High Spatial and Temporal Resolution in Atomic Force Microscopy

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Atomic Force Microscopy in vacuum readily achieves atomic resolution however imaging in fluid has substantially lower resolution due to the damping of the cantilever by the fluid. We reduced the damping and force noise of the probe by building a protective encasement around the cantilever which keeps the cantilever dry but allows the tip to probe the sample in solution. The substantially lower viscosity of the air leads to orders of magnitude improvement in force noise. Furthermore, encased cantilevers retain high resonance frequencies and Q factors when immersed in liquid such that they are thermally limited even at high stiffness. This enables high resolution imaging and force spectroscopy on all commercial AFM systems in liquid.

Raster scanning is not ideal for high speed AFM. Less than half of the scan time is used to create an image because trace and retrace can not be interlaced due to piezo hysteresis. Furthermore, the scanner resonance distorts triangular waveforms or requires sinusoidal scanning, where images are rendered from even less than half of the scan time. using advanced image processing tools, such as Inpainting, enables image rendering from 100% of the scan time and use of scan waveforms that best match the inertial limits of the scanner. We have also developed a Z piezo with over 300 kHz bandwidth enabling high scan speeds frame rates. Spiral scanning together with encased cantilevers will lead to significant advances in temporal and spatial resolution for soft biological samples imaged by Atomic Force Microscopy.

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Addressing the Inverse Problem of Far-Field Imaging: A Noniterative Exact Solution for Phase in Imaging

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This talk describes an approach to a solution of inverse problems in far-field imaging with application to optical, electron/ion beam, x-ray and other imaging modalities. Two aspects of the inverse problem in optical imaging are phase and resolution. Whereas techniques such as STED, PALM, STORM etc address the latter, they lack phase information as does near-field optics. Furthermore, all of these techniques are serial. We introduce an approach based on a controllable nanoscopic far-field optical point source integrated into parallel imaging. In the first demonstration of this development we focus on the inverse problem of the phase of an image which has never been solved exactly and can only be approached through iterative methods with all their problems of nonconvergence, slow convergence, convergence to local minima, and stagnation. We now show that it is possible to obtain, with such a method, an exact solution to the inverse problem of phase both experimentally and theoretically. Our method is based on the breakthrough that crystallography experienced in phase retrieval for large molecular entities by Max Perutz's introduction of ''heavy atoms'' using the method of isomorphous replacement. Scanning probe microscopy and its full integration with optical microscopy allows us to apply these X-ray concepts to implement ''heavy atom'' restoration of phase in optical phase retrieval. In analogy to the heavy atom method, we acquire Fourier intensities in place of an X-ray diffraction pattern, and in place of the heavy atom, we utilize a nanometrically translatable point source of light, coherently related to the far-field illumination this leads to 3D optical imaging. The methodology has super-resolution potential, and thus, heavy atom restoration of phase with super-resolution (HARPS) has the potential to provide super-resolution 3D images in real time.

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Demonstration of High Speed AFM through Local Raster Scanning

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Efforts in high-speed atomic force microscopy (HS-AFM) continue to improve the rate of imaging in AFM. There remain, however, many systems of interest whose speeds are far beyond the fastest systems, such as the motion of dynein walking along a microtubule. Unlike traditional approaches to HS-AFM, our work seeks to take advantage of a prior knowledge about the sample to achieve gains in the imaging rate. To date we have been focusing on string-like samples such as biopolymers and have developed the Local Raster Scan algorithm (LRS). The algorithm uses the data acquired by an AFM in real time to steer the tip so that it stays on a sample of interest. As a result, the imaging rate is increased by directly decreasing the number of samples. The basic idea can also be used to achieve even better temporal resolution by abandoning imaging in favor of direct tracking of the motion of a macromolecule. The algorithm has been implemented on a commercial AFM (Agilent 5500) and demonstrated on both linear and circular grating samples. Comparisons to standard raster scanning (with equivalent settings) show that a reduction of at least an order of magnitude in imaging time can be achieved. These experiments also highlight challenges arising from the feedback nature of the LRS algorithm.

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The Mechanical Properties of Modified Fibrin Fibers: Blocking the B-b Knob-Pocket Interaction

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Fibrin clot formation has been studied to determine the mechanical properties of fibrin fibers modified by blocking the B-b knob-pocket interaction. Synthetic B-knob peptides AHRPYAAC or AHRPYAAC-Peg have been added to a fibrinogen solution to allow for binding to the b-pockets prior to clot formation. After fibrin clot formation, a combined atomic force microscopic (AFM)/optical microscopic technique was used to study the properties of individual fibrin fibers in buffer. Mechanical testing of fibers was done using the AFM to laterally stretch individual fibers suspended over 13.5μm wide groves in a transparent substrate. The optical microscope, located below the sample, was used to monitor the stretching process. We found that the density and lateral aggregation of fibers was hindered by blocking the b-pockets with the synthetic B-knobs. Fibrin fibers modified with the synthetic peptide AHRPYAAC-Peg were found to stretch to 2.27 times their original length before rupturing